

**Cytological Observations on the Early Stages
of Segmentation of Echinus Hybrids.**

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With Plates 28 and 29.

INTRODUCTION.

THE material on which the greater part of the observations here described was made was handed over to us by Messrs. Shearer, De Morgan and Fuchs, and was collected by them while carrying out experiments on the characters of the plutei in hybrids between the British species of *Echinus*. A preliminary account of their results has been published,¹ and their fuller paper appears concurrently with this. In view of the fact that in some of the hybrids the plutei, whichever way the cross was made, were essentially of the maternal type, it was clearly of interest to determine whether an elimination of paternal chromosomes takes place comparable with that described by Baltzer,² who has found that in the early stages of hybrids between different Echinoid genera, paternal chromosomes are eliminated in those cases in which the plutei are of the maternal type. The species used in the present experiments were *Echinus esculentus*, *E. acutus*, and *E. miliaris*, the three species of *Echinus* found at Plymouth, where the experiments were made. The material obtained

¹ 'Journ. Marine Biol. Soc.,' ix, 1911, p. 121.

² 'Arch. f. Zellforsch.,' v, 1910, p. 497.

in 1911 consisted of segmenting eggs of these three species, and of the hybrids *esculentus* ♀ × *acutus* ♂, *acutus* ♀ × *esculentus* ♂, and *acutus* ♀ × *miliaris* ♂, preserved at intervals of from half an hour to five hours after fertilisation.

In 1912, besides a few additional batches of the same crosses, we obtained eggs of *esculentus* ♀ × *miliaris* ♂ and *miliaris* ♀ × *esculentus* and *acutus* ♂. The great majority of the eggs sent to us were in stages from shortly before the conjugation of the pronuclei up to 2- or 4-cell stages; very few had reached the third segmentation division. Several samples of eggs were from batches the remainder of which were reared to plutei.

The preservatives used were sublimate-acetic, sublimate-nitric, Flemming's, Hermann's and Perenyi's fluids. The preservation varied in different cases; in general, sublimate-acetic and Flemming gave the best results. The eggs were sectioned (the sections we made ourselves were 5 or 7 μ in thickness), and stained on the slide with Heidenhain's iron-haematoxylin. In counting chromosomes we have found it absolutely necessary to draw every spindle; repeated attempts to count by eye have shown that by this method a number smaller than the true one is usually recorded.

Before describing the hybrid eggs, a few words are needed on those of the pure species. The greater part of the work has dealt with crosses between *acutus* and *esculentus*; of pure *miliaris* comparatively little material was available, and an account of it will be postponed until the *miliaris* hybrids are dealt with. Of *acutus* and *esculentus* we had a considerable supply of material from the early stages of fertilisation up to the second segmentation division. No account is needed of the stages of fertilisation nor of details of the segmentation mitoses except those relating to the chromosomes. We have determined the chromosome number in both species as 38; in early anaphase groups seen in face this number can frequently be counted with confidence (Pl. 28, figs. 1, 2, 3), and where a lower number is found it is

probable that some chromosomes are missing or concealed. The chromosome groups of the two species are very much alike; we have spent a considerable time in trying to discover points of difference which might be used in the study of hybrid eggs, but have failed to find any that are trustworthy. The chromosomes differ considerably in size and shape; in early anaphase figures seen in side view the following points are usually recognisable. (Fig. 3): Two chromosomes are noticeably longer than all the rest, and commonly complete their division slightly later than the remainder. Two are U- or V-shaped; if seen with the two limbs almost superposed they are recognisable by their apparently greater thickness. About four are longer than the remainder, though shorter than the two long ones mentioned above, and are not usually hooked at the ends; these, however, are not always distinguishable from the somewhat shorter rod-shaped chromosomes. The remainder are about equally divided between bodies the length of which is several times as great as their width, and shorter rods which often appear as oval or round dots if seen slightly obliquely. The chromosomes of both the last classes are frequently hooked at the end towards the pole; sometimes this is so pronounced as to cause them to resemble the V-shaped bodies mentioned above. Since the various classes described grade into one another almost imperceptibly, and are so similar in the two species, we have not been able to distinguish paternal and maternal chromosomes in the hybrid eggs in the crosses between *acutus* and *esculentus*.

The eggs of the pure species differ from those of the hybrids in the greater uniformity of the rate of development. In any batch of eggs of *acutus* or *esculentus* the majority are in stages of development not very far removed from one another, though there is not absolute uniformity. In the hybrid eggs, however, there is great diversity, and this appears to be the case to about the same extent whichever way the cross is made. In batches of which some eggs have reached the four-cell stage, others will show prophases of the

first division, or stages of conjugation. A considerable proportion of the eggs is also usually not fertilised.

THE HYBRIDS BETWEEN *ESCULENTUS* AND *ACUTUS*.

(i) *Esculentus* ♀ × *acutus* ♂.

In the eggs of this cross the behaviour of the nuclei appears to be perfectly normal. The conjugation of the pronuclei differs from that in *acutus* ♀ × *esculentus* ♂ described below in the fact that the sperm-nucleus often reaches a size nearly equal to that of the egg-nucleus before the two begin to unite, and that chromosomes are visible in both nuclei before conjugation. The mitotic figures are like those of the parent species, quite regular in every respect as far as the second segmentation division, and no trace could be found of chromosome elimination. The chromosome number in the first and second divisions appears to be 38 as in the parent species; elimination, if it occurs at all, does not take place until a later stage (Pl. 28, figs. 4, 5).

(ii) *Acutus* ♀ × *esculentus* ♂.

The mitotic figures in eggs from this cross differ conspicuously from those of the converse cross or the parent species, and it is to them that the greater part of our attention has been devoted. The conjugation of the nuclei appears to take place without serious abnormality. We have many examples of the two nuclei lying in contact, the sperm-nucleus distinguished by its smaller size and conspicuous central mass of chromatin. Rather later stages show that the sperm-nucleus becomes absorbed into the egg-nucleus before this chromatin mass breaks up, while the male nucleus is still quite small, and before the chromosomes of the egg-nucleus are formed; the rather elongate zygote nuclei with an aster at each end, such as are common in pure *acutus* eggs and in the cross, *esculentus* ♀ × *acutus* ♂, are not

usually found. In pure *esculentus* the process of conjugation appears to be more like what we have found in the *esculentus* ♂ hybrids. The zygote nucleus swells, irregular masses of chromatin appear within it, and become concentrated into small elongated chromosomes, the number of which is about thirty-eight. As the nuclear membrane disappears, and the spindle is completely formed, the chromosomes, at this stage scattered irregularly, become shortened and thickened, and often clumped in pairs or groups, so that a count usually gives a number much lower than that at rather earlier or later stages. Sometimes, however, they are scattered more separately, so that each can be seen distinctly, and here again thirty-eight may be counted (fig. 6, *a, b*). At about this stage, however, the abnormal feature of the mitosis appears; small vesicles of varying size and number appear among the chromosomes, distributed irregularly on the spindle (figs. 7-11). In eggs preserved with osmic fixatives these vesicles are faintly and evenly stained, except that the edge is more deeply stained than the centre; in sublimate preparations they have more the form of minute nuclei with small stained dots under their enclosing membrane, and are rather similar to the vesicles normally formed by the chromosomes in the late anaphase as they cluster round the pole of the spindle, although quite distinct from them.


A comparison of a series of prophase figures, or even of the vesicles in one figure, leaves no doubt that they are formed from chromosomes, for all stages may be found between a chromosome which appears somewhat swollen and a fully formed vesicle (figs. 7, 8, *a-z*). But it is also quite clear that in many cases the whole chromosome is not used up in forming the vesicle; often apparently normal chromosomes may be seen to which a vesicle is attached, either at its side or at one end. The number of vesicles which are formed at this stage varies from one or two up to about six or seven, or occasionally even as many as twelve, as shown in the counts in Table I. The considerable variation in the number of chromo-

some and of vesicles is due in part to the fact that the vesicles are being formed at the stage at which the counts were made, and in some nuclei others might still have been produced; and as regards chromosomes, to the fact that at this stage they show a strong tendency to become clumped. The counts in Table I were made by eye; the number of vesicles in each case may be regarded as trustworthy, of chromosomes only approximate.

TABLE I.—Numbers of Chromosomes and Vesicles in Prophase Figures (first division), acutus ♀ & esculentus ♂.

Chromosomes.	Vesicles.	Chromosomes.	Vesicles.
31 .	12	29 .	6
29 .	9	35 .	2
32 .	6	32 .	2
31 .	2	29 .	3
33 .	3	31 .	0
28 .	1	32 .	3
33 .	1	32 .	4
31 .	6	32 .	7
28 .	2	31 .	3
31 .	3	32 .	3
		33 .	5

We at first believed that each vesicle was formed from a chromosome which was entirely used up in the process, but a detailed examination and comparison of different stages makes this view untenable. We believe that the vesicle formation may be explained as follows: In the late prophase stages, when the normal chromosomes are beginning to split longitudinally, some of the chromosomes, instead of splitting, tend to swell up and form vesicles as the normal chromosomes do in the late anaphase stage. Every gradation may be found between chromosomes which behave normally and those which are completely converted into vesicles.

Some merely become swollen and faintly stained in the centre; others develop a vesicle at one side or end while the rest of the chromosome appears normal; others, again, split more or less completely, but a vesicle is formed by one or possibly both of the longitudinal halves. Whether a chromosome of which one half forms a vesicle divides completely or remains undivided in anaphase, seems to depend to some extent on its position on the spindle at the time when the vesicle develops. Before the equatorial plate stage the chromosomes are scattered quite irregularly over the spindle, but already may show traces of the longitudinal split (fig. 9, *a, b*). If at this stage one half of a chromosome develops a vesicle, it prevents the whole chromosome from reaching the equatorial plate, with the result that both halves of the chromosome are included in the anaphase group belonging to the pole of the spindle to which the chromosome happened to be nearer in the prophase. This is especially well seen in the case of one chromosome which is conspicuously longer than the rest. Two such long chromosomes are found in both *esculentus* and *acutus*; the hybrid also has two, one doubtless derived from each parent. In the pure species these long chromosomes complete their division rather later than the smaller ones, making bracket-shaped figures, , just before the complete separation of the halves. In the hybrids it may sometimes be seen that one of them is in this stage in the middle plane of the spindle, while the second, half of it bearing a vesicle, lies between the equator and one of the poles (fig. 18*b*).

At the close of the prophase the normal chromosomes arrange themselves in an equatorial plate, and are at this stage rather long and bent. Some of them are already completely transformed into vesicles; to some, vesicles are attached, and probably others have already formed and thrown off a vesicle. The vesicles may be included in the equatorial plate, or remain scattered on the spindle; some are nearly always left in the equatorial plane just outside the spindle, and take no further part in the division. The chromosomes now divide along the split of which traces can

be seen in earlier stages, and the halves pass to the poles. We have already pointed out that in some, to which vesicles are attached, this division is incomplete and both halves probably pass to the same pole. There are occasional indications also that sometimes a chromosome which has not actually become vesicular, but which shows traces of swelling, may fail to divide, and pass entire to one pole. For this reason, in some early anaphases, while the two halves of most of the chromosomes are easily recognisable, some appear to be without mates, and the numbers in the two daughter-groups are not always equal. The numbers of chromosomes and vesicles found in anaphases of the first division are given in the counts recorded in Table II, which are all made by drawing. The numbers bracketed together indicate the two daughter-groups of one spindle; the total number of vesicles is given by the first figure, that of typical, unshrunk vesicles by the figure in round brackets ().

TABLE II.

Chromosomes.	Vesicles.	Chromosomes.	Vesicles.
32-35 } . .	9 (5)	36 } . .	5 or 6
33-35 } . .		30-32 } . .	
34-36 } . .	4	70-75† . .	1
35 } . .		30-32 } . .	
31-34 } . .	5	32 } . .	2
33-36 } . .		37 } . .	
29* } . .	0	30-31 } . .	7
30* } . .		37 } . .	
		35 } . .	5
		64-65† . .	

* Numbers approximate.

† Daughter-groups not distinct enough to count separately.

The exact number of vesicles in anaphase is difficult to determine, for while some of them appear to have increased in size, others appear to have shrunken and become reduced to

round, deeply stained bodies, which are not always easy to distinguish from large yolk-granules or sometimes from normal chromosomes. Every stage may be found between these stained dots and true vesicles, and in the telophase the normal chromosomes within the daughter-nuclei give rise to chromatic dots exactly similar in appearance. The fate of the vesicles depends upon their position on the spindle; those which lie among the chromosomes of the anaphase groups are carried with them to the poles and become included in the daughter-nuclei, while those which lie on the periphery of the spindle or just on its equator remain where they are, and are excluded from the nuclei. They may commonly be found along the line of the cell division in the 2-cell stage, already considerably shrunken and reduced to stained dots. The number eliminated as counted in late anaphase figures before the cell-division varies from none to about five, but in 2-cell stages a larger number of chromatic dots may sometimes be seen, and it is possible that they break up as they undergo degeneration. Figs. 10-14 illustrate anaphase and telophase stages of the first segmentation division.

Before proceeding to describe the second segmentation mitoses we must refer to a curious phenomenon which is very common in the first division figures. In a very large proportion of anaphases of the first division, and not rarely before the chromosomes have become arranged in the equatorial plate or even immediately after the dissolution of the nuclear membrane, it is seen that the centrospheres of the spindle have become divided, so that what at first appears as a bipolar or more commonly quadripolar spindle results. The extent of the division varies; the two halves of the sphere may be close together so that the spindle-fibres are not seriously deranged (figs. 10, 11), or they may, even in prophase, be widely separated, and a small secondary sheaf of spindle fibres may sometimes be seen between them (figs. 15, 16). At first we took these spindles with divided poles to be multipolar spindles caused by the entrance of more than one

spermatozoon, but an examination of a series shows that every stage occurs between normal spindles, through those with poles just divided to examples with four, or sometimes three quite distinct spheres to which the fibres converge.¹ A further difference is that in no case have we found a noticeably abnormal number of chromosomes; sometimes they appear to be considerably below the normal number, due doubtless to "clumping," but never conspicuously above the number expected. In one batch of eggs of pure *esculentus*, on the other hand, true tripolar and quadripolar spindles are of very frequent occurrence, in fact hardly any of the eggs are dividing normally; but in this case the chromosome number is constantly about either one and a half times or twice the normal (38). For example, in this batch of pure *esculentus* four counts of chromosomes on abnormal spindles (late prophase) gave respectively 52, 70, 71 and about 65 chromosomes (the numbers are only approximate); numbers of this kind have never occurred in the *acutus* ♀ × *esculentus* ♂ hybrids. We can only conclude, therefore, that in eggs of this cross there is a tendency for the centrospheres to divide prematurely, but the daughter-spheres rarely separate so widely as to cause an abnormal cell division. A moderate division of the spheres has been seen in some anaphase figures of pure *acutus* and of the converse cross *esculentus* ♀ × *acutus* ♂, but in these eggs it is never so extensive as in the cross *acutus* ♀ × *esculentus* ♂.

In the prophase of the second segmentation mitosis the chromosomes appear within the nucleus as elongated, more or less bent rods, like those of the same stage in the first division, but the smaller size of the nuclei and frequent interlacing of the chromosomes make trustworthy counting very difficult. Within the nucleus sometimes a varying number of faintly stained, round bodies may be seen, which we at first took for vesicles which had been included in the nucleus in the first telophase, but the inconstancy of their occurrence

¹ Boveri has figured similar divided poles in *Echinus microtuberculatus* ('Zellenstudien,' iv, Taf. v, fig. 59).

makes this interpretation very doubtful. When the nuclear membrane dissolves the figures are closely like those in the first prophase; the chromosomes become scattered over the spindle, and, as before, some of them are seen giving rise to vesicles (fig. 17, *a, b, c*). These differ from those of the first segmentation mitosis only in being in general somewhat smaller, and in the fact that complete chromosomes appear less often to become entirely vesicular; the vesicles seem rather to be formed on the ends or sides of the chromosomes, and often quickly become separated from them. The fact that the average number of vesicles in the second division is about the same as that in the first, and that the chromosome number, as counted in anaphase, shows no further diminution, suggests that the same chromosomes which produced vesicles in the first mitosis again do so in the second.

The division of the poles of the spindle is less conspicuous in the second segmentation mitosis; in anaphase they are often elongated transversely to the axis of the spindle, or divided to a small extent, but exactly similar figures are not uncommon in pure *esculentus* eggs in the same stage. The vesicles, as before, are scattered among or outside the chromosomes, and in anaphase some are included among the daughter groups and enter the nucleus of the 4-cell stage; others remain in the centre or round the edges of the spindle and are eliminated (figs. 18, 19, 20).

Counts of late prophase and anaphase groups gave the results recorded in Table III; the prophase figures were counted by eye, the anaphases by drawing. As in the case of the first division the anaphase figures are more trustworthy. Brackets on the right side of the numbers indicate anaphase groups of the same spindle; on the left side spindles of the same egg.

TABLE III.—Counts of Chromosomes and Vesicles in Second Divisions.

PROPHASES.		ANAPHASES.			
Chromosomes.	Vesicles.	Chromosomes.	Vesicles.	Chromosomes.	Vesicles.
{ 33	. 6	34-35}	4	{ 37	5
{ 35	. 3	35 }		{ 37	
35	. 6	35}	7	{ 35*	6
{ 28	. 6	35}		{ 35	
{ 29	. 8	{ 35 }	6	{ 36 }	7
32	. 2			{ 35 }	
31	. 1	{ 35 }	6	{ 38 }	6
{ 32	. 4	{ 36 }		{ 35 }	
{ 32	. 3	{ 33 }	6	{ 33-35 }	5
{ 30	. 5			{ 35 }	
{ 32	. 3	{ 35 }	5	{ 34-35 }	4
{ 30	. 3	{ 35 }		{ 34 }	
{ 31	. 5	36}	8	{ 71-72†	5
		35}		{ 31-34 }	
		35-36}	5	{ 33 }	6
		33-34}		{ 31 }	
				{ 34 }	5
				{ About 30*	1

* In these figures the chromosomes had not yet divided.

† Two ends of spindle not accurately separable: two or three chromosomes possibly cut in two sections.

It will be seen that while there is considerable variation in the chromosome number, the numbers in the two spindles in most eggs are in fair agreement, and further, that the number of vesicles added to the chromosome number is often considerably above 38. This, and the fact that there is no diminution in the chromosome number as compared with that in first division anaphases, confirms us in our belief that in the second division the vesicles are given off from chromosomes rather than that whole chromosomes are converted into vesicles. Occasionally also in second division anaphases, chromosomes which have clearly divided, and are travelling

in a normal way to the poles, are seen to have vesicles attached to them. We conclude, therefore, that the tendency to vesicle-formation is diminished in the second division, and that nearly all the chromosomes which entered the nucleus of the 2-cell stage are able to divide and move to the poles in the normal way. Our material does not provide any examples of segmentation divisions later than the second in the cross *acutus* ♀ × *esculentus* ♂, but we defer any discussion of the facts observed until the hybrids with *E. miliaris* have been described.

E. MILIARIS AND ITS CROSSES WITH *ACUTUS* AND *ESCULENTUS*.

The material obtained in 1911 included only one cross with *E. miliaris*, viz. *acutus* ♀ × *miliaris* ♂. Unfortunately the supply of pure *miliaris* eggs was small and not very good, so that we cannot give so full an account of it as of the other species. All the eggs showing mitotic spindles in pure *miliaris* were in the 4-cell stage or later, and owing to the small size and crowded state of the figures counting was difficult. The chromosome number appears to be smaller than in the other species; many counts, especially of equatorial plates, gave numbers ranging from 30 to 32 or 33, but in two anaphase groups in one spindle, in which the chromosomes were very clearly shown in face, 34 may be counted at each pole (fig. 21), and this number is confirmed by counts of the cross *esculentus* ♀ × *miliaris* ♂ (1912). The chromosomes are more nearly alike in size than in *esculentus* and *acutus*.

Acutus ♀ × *miliaris* ♂.

In the eggs from the cross *acutus* ♀ × *miliaris* ♂ the spindles differ noticeably from those of the *acutus* and *esculentus* crosses (figs. 22-25). The spheres with their radiations are very large and conspicuous, and only rarely show any division. The spindles are much narrower, with the

result that the chromosomes are more crowded; this relative narrowness may be due partly to the absence of divided poles, which in the *esculentus* hybrids cause considerable widening of the whole figure, but this cannot completely account for the difference, for in the exceptional cases of divided poles in the *miliaris* cross, the spindles are still very narrow. The general appearance of the spindles and asters is much more like that of pure *miliaris* than of *acutus*, suggesting that the centrosome introduced by the *miliaris* spermatozoon can cause mitotic figures of the *miliaris* type to develop in *acutus* eggs. Our observations on the hybrid eggs have been made almost entirely on two lots, which gave in some respects dissimilar results. One lot consists of stages from the beginning of the first to the beginning of the second segmentation division, all the stages of the first division being well represented. The second lot contains much more advanced eggs, up to the 16-cell stage at least, but also includes early stages from prophases of the first segmentation division onwards. In a preliminary examination of the first lot we failed to find any trace of elimination or of vesicle formation; both equatorial plates and anaphases were somewhat irregular, but the conspicuous vesicles found in the *acutus* ♀ × *esculentus* ♂ cross appeared quite absent. In the second lot, however, vesicles were not rarely found in the earlier divisions, and when present were of the same kind and as conspicuous as in the cross with *esculentus* ♂. A re-examination of the second lot showed that vesicles were sometimes present, but were in most cases very small, and attached to chromosomes as in prophases of the *acutus* ♀ × *esculentus* ♂ cross (fig. 22). Further, in metaphase and early anaphase it is sometimes, though not by any means always, possible to see that a few (one to three) chromosomes are swollen, irregular in shape, and without any trace of a division at a time when the halves of the remainder are beginning to separate. In later anaphases the majority of the chromosomes are rod-shaped, long and narrow, but sometimes a few are much larger and more ovoid in shape. In anaphases occasionally a minute vesicle may be seen left on the spindle,

but these are always so small and faint as to be recognisable with difficulty, and in telophase and the 2-cell stage no trace of elimination is to be found.

In the second lot of eggs typical though small vesicles are commonly to be found in the first division, and may be seen, though with progressively less frequency, in the second, third and sometimes the fourth. The vesicles in these eggs are in most cases still attached to chromosomes, and are included with them in the daughter-plates, and appear to be carried into the nucleus. Vesicles are rarely if ever left on or outside the spindle as in the first and second divisions of the cross with *esculentus*. The interpretation of these eggs is made difficult by the fact that those in the earlier stages are almost all abnormal; many have multipolar spindles and nuclei dividing without division of the cytoplasm, and frequently there is evidence of polyspermy. The later stages of this batch, however, from the 8-cell stage onwards, appear to be normal, and an occasional vesicle may be found in them also. We suggest that the behaviour of the eggs in the two lots may be reconciled as follows: In the first divisions of the cross *acutus* ♀ × *miliaris* ♂ a small but varying number of chromosomes may show a tendency to form vesicles as in *acutus* ♀ × *esculentus* ♂; in normally developing eggs the vesicles are usually small and do not become detached from the chromosomes, but when the tendency to form vesicles is present the chromosomes affected probably fail to divide and are carried entire to one or other pole. In eggs the development of which is delayed and which are abnormal through polyspermy or other causes, larger and more conspicuous vesicles are formed, which, however usually remain attached to the chromosomes and so are not eliminated from the daughter-nuclei. The non-division of some of the chromosomes is not an easy matter to prove, and in many eggs all appear to behave quite normally, but that in some eggs one or more fail to divide is suggested by three facts: (1) The great difference in thickness among the chromosomes in some anaphases suggests that some of them have failed to divide.

(2) In early anaphase it is often possible to see which chromosomes in the daughter-groups correspond with each other, and it is usually the swollen and irregular ones which appear to be without mates; unfortunately, however, the narrow and crowded spindles make the determination of this point uncertain in many cases. (3) The third piece of evidence is from counts of the chromosome number. If *acutus* has 38 and *miliaris* 34 chromosomes, the hybrid should have 36. We have found two eggs in which the spindle is replaced by a monaster (fig. 26), and in these the chromosomes, though some of them are constricted, have not divided, and in each 36 can be counted, in one case with confidence, in the other with great probability.¹ Prophases after the nuclear membrane is dissolved are in this cross usually too crowded for counting, but we have found one in which about 36 are also present. In almost all other counts of both batches of eggs

TABLE IV.—Chromosome Counts in *Acutus* ♀ × *miliaris* ♂.

Prophases and Metaphases.	Anaphases.
36 (39?) (monaster)	36 }
36 + 1 small vesicle (monaster)	34 }
32 (35?) + 1 vesicle }	35
36 + 1 ,, }	35
31* + 1 ,, }	35 (33-36)
29* + 1 ,, (?) }	35 }
34 }	32 (33?) }
37 }	34 }
30-34 + 1 vesicle }	35 }
34-35 }	35-37 }
	33-55 }
	31 }
	37 }

* These numbers are probably too low.

¹ That the number when no vesicles are present should be 36 is further indicated by the similar cross *esculentus* ♀ × *miliaris* ♂ (see below), in which the number is almost certainly 36.

the number is lower, as is shown in Table IV. The two ends of one spindle are bracketed as before.

The small size of the chromosome groups, with the resultant crowding, doubtless makes the numbers in Table IV lower than the true figure, and further, when the chromosomes are in very close proximity, it is always possible for two of them, if the fixation is not very perfect, to become so united that they appear as one of double thickness. In the pure *miliaris* eggs, although we have sections in which 34 chromosomes can be counted with little doubt, the majority of counts gave from 31 to 33, and hence we are not disposed to lay very great weight on the counts in the *miliaris* hybrids. Nevertheless the frequency with which the counts fall below the theoretical number 36, coupled with the facts that vesicle-formation on a small scale is not infrequently seen in the early stages of division, and that in early anaphases swollen chromosomes without visible mates at the other pole are also sometimes found, suggests that in some eggs at least a reduction in chromosome number may be brought about by non-division of one or more chromosomes.¹

It appears, therefore, that in the *acutus* ♀ × *miliaris* ♂ cross a small number of chromosomes show a varying tendency to swell and form vesicles in the early stages of the first division; when the tendency is pronounced this may cause failure to divide in the metaphase, and they become carried to one or other pole. Exceedingly small vesicles are sometimes eliminated, but this process is not frequent and conspicuous as it is in the cross *acutus* ♀ × *esculentus* ♂. In the later divisions the abnormal tendency is less marked, and by the third or fourth segmentation

¹ A somewhat cursory examination of three batches of eggs of *acutus* ♀ × *miliaris* ♂, obtained shortly before going to press, confirms our account of this hybrid. The eggs, almost all of which are fertilised, are chiefly in the later stages of the first division. The spindles are like those obtained in 1911; most of the figures are normal, but a few show very small vesicles, and one case was found of a large vesicle eliminated in the telophase.

division all the chromosomes have usually regained their normal behaviour. When widely different numbers are counted at the two poles of one spindle in the later segmentation divisions (cf. fig. 27 *a, b*), it is possible that some chromosomes are still behaving abnormally, but another possible explanation is that owing to the irregularity of the equatorial plate the two halves of the chromosomes are still seen in one daughter-plate.

Comparing these conclusions with what has been described in the cross *acutus* ♀ × *esculentus* ♂, it is seen that in the latter cross a similar tendency is more strongly shown; some chromosomes swell up entirely to vesicles which are left out of the spindle, and others produce smaller vesicles, and in some cases probably fail to divide. The vesicles which are left near the equator of the spindle are eliminated. In the second division there is already evidence that the chromosomes are beginning to recover, and the results obtained from the cross *acutus* ♀ × *miliaris* ♂ suggest that in the later segmentation divisions their behaviour would again be normal. Owing, however, partly to the elimination of some entire chromosomes in the first division, and to the failure of others to divide, the chromosome number of the *acutus* ♀ × *esculentus* ♂ hybrid would be reduced from 38 to somewhere between 31 and 37, the number varying in different embryos, and probably sometimes in different cells of the same embryo.

The preceding account deals with the hybrid eggs obtained in 1911. In that year, owing to an oversight, no eggs of suitable age were obtained from the crosses *esculentus* ♀ × *miliaris* ♂, and of *miliaris* ♀ × *esculentus* and *acutus* ♂. These hybrids were obtained in 1912, but, as Shearer, De Morgan and Fuchs have recorded,¹ the plutei obtained from them differ considerably from those reared in 1911, and the *miliaris* eggs especially appear in this year to develop much less satisfactorily than in 1911. It cannot,

¹ 'Nature,' June 27th, 1912.

therefore, be concluded with complete confidence that our results obtained from these hybrids are entirely typical.

Esculentus ♀ × *Miliaris* ♂.

The eggs of this cross preserved in 1912 were mostly in the 2-cell stage, but a considerable number also show stages in the first division. Almost all are fertilised and in general are developing quite normally. No elimination of chromosomes nor formation of vesicles was observed, and the mitotic figures are in most cases as regular as in the eggs of the pure species. The spindles are less noticeably narrow than those described in the cross *acutus* ♀ × *miliaris* ♂, but are probably narrower than in pure *esculentus* eggs or in *esculentus* ♀ × *acutus* ♂; divided poles are not found. Counts of chromosomes leave little doubt that the number is 36. Seventeen counts of anaphase groups, in 11 eggs, gave 36 in nine cases (7 eggs), including nearly all the most satisfactory figures; 37 in two cases in which a pair, counted as two, are probably really one V-shaped chromosome; and in six counts either 34 or 35. Some of the cases in which 36 are seen are so clear that there can be little doubt that it is the true number (fig. 28). If our estimate of 34 for *miliaris* and 38 for *esculentus* be correct, 36 is the number to be expected. One V-shaped chromosome is commonly visible; there are two in *esculentus*, and none (probably) in *miliaris*. In a considerable number of the anaphase figures (about eight out of twenty examined on this point), no V could be found, and for a time we thought that this body might be comparable with the hook-shaped chromosome found by Baltzer in half the eggs of *Strongylocentrotus* and the horse-shoe found in half those of *Echinus microtuberculatus*.¹ Further study, however, has convinced us that in our material it is almost impossible to determine with certainty the presence or absence of this element; when seen sideways it is practically indistinguish-

¹ 'Arch. f. Zellforsch.,' ii, 1909, p. 549.

able from one of the thicker rods, and it may be present in one cell of a 2-cell stage, and apparently absent in the other.

Miliaris ♀ × Esculentus ♂ and Miliaris ♀ ×
Acutus ♂.

These two crosses, the converse of the last two described, may be taken together. As has been mentioned above, the miliaris eggs in 1912 were very unsatisfactory, only a small proportion yielding larvæ even when fertilised by their own sperm. In our material of the hybrid eggs, in both cases an exceedingly small proportion showed evidence of fertilisation. In the cross with esculentus ♂ about 2 per cent. are beginning to develop; in that with acutus ♂ the percentage is considerably lower. Most of the developing eggs in both cases are in the 2-cell stage, but nearly all stages of both first and second segmentation divisions have been found, and at least one clear case of the conjugation of the egg and sperm nuclei.

The figures in both crosses are distinguished from those of the converse crosses by the greater irregularity of the chromosomes on the spindle. The extent of this irregularity varies; some figures are almost normal, in others the chromosomes are much scattered, but cases are rarely found in which an accurate count of chromosomes is possible. In one unusually regular first anaphase of miliaris ♀ × acutus ♂ cut transversely to the spindle, 35 may be counted at one end and about 36 at the other; in this case, however, four chromosomes are scattered entirely outside the main group around the pole. In late anaphases and telophases of both crosses it may often, but not always, be seen that one, two, or probably sometimes more, chromosomes lag to such an extent that they are not included in the daughter-nuclei (figs. 29, 30). In some cases they appear not to divide; in others the halves separate so late that they are left behind in the cytoplasm. Sometimes this causes the daughter-nuclei to have a tail-like

projection extending towards the line of cell-division; in others the daughter-nuclei are normal, but a stained mass of chromatin remains in the cytoplasm on the line dividing the two cells. In 2-cell stages such a chromatic mass, usually very small, but of varying size, is very commonly found pressed against the faces by which the cells are in contact, sometimes in only one of the daughter-cells, at others in both. In other eggs no sign of elimination is found, but it is possible that in some at least of these the eliminated chromosome has been absorbed.

The second division figures in our material are mostly in metaphase or early anaphase, so that it is difficult to determine with certainty whether any chromosomes are eliminated in them; in one case in an anaphase one chromosome appears not to be dividing (*miliaris* ♀ × *acutus* ♂), but in the few other cases observed no evidence of elimination in the second segmentation division was found. The spindles in these crosses, especially in the second division, are very short, with poles near together; not very infrequently nuclear division occurs not followed by cell-division.

In general, then, it may be concluded with regard to the *miliaris* crosses, that in (1) *acutus* ♀ × *miliaris* ♂ a small but variable number of vesicles, such as we have described in *acutus* ♀ × *esculentus* ♂, is sometimes formed, and that the chromosome number is possibly reduced thereby to a small extent. (2) In *esculentus* ♀ × *miliaris* ♂ there is no abnormality, and the observed chromosomal number is that which is to be expected from our determination of the numbers in the parent species. (3) In the converse crosses *miliaris* ♀ × *acutus* and *esculentus* ♂, no vesicles are produced, but in the anaphase of the first division one or more chromosomes commonly either fail to divide, or divide so late that they are not included in the daughter-nuclei.

SUMMARY AND DISCUSSION.

Our work was undertaken primarily in order to discover whether in the crosses described there was any systematic elimination of chromosomes, such as have been found by Baltzer in crosses between *Sphærechinus* and *Strongylocentrotus*, which might be correlated with the facts described by Shearer, De Morgan and Fuchs in their work on the hybrid plutei. Baltzer found that in the cross *Sphærechinus* ♀ × *Strongylocentrotus* ♂ no elimination of chromosomes occurs, and the hybrid plutei are intermediate between the parental types. In the converse cross (which is successful in a very small percentage of cases), sixteen chromosomes, regarded as belonging to the male (*Sphærechinus*) parent are eliminated, and the hybrid plutei, when exceptionally they can be reared, are of the maternal type. In our cases, Shearer, De Morgan and Fuchs have found, in the years preceding 1912, that in crosses between *esculentus* or *acutus* and *miliaris* the plutei, in certain characters at least, were of the maternal type, whichever way the cross was made. It seemed possible, therefore, that we might find elimination of chromosomes in the manner described by Baltzer.

For several reasons the work described above has followed somewhat different lines from those expected at the beginning. In 1911, the only cross with *miliaris* which was obtained before the season was too far advanced was *acutus* ♀ × *miliaris* ♂. In the hybrid eggs we found that some of the chromosomes developed vesicles, but no other elimination occurred except the possible non-division of certain chromosomes, about which we are uncertain. The vesicle formation, which appears to a small extent in eggs of *acutus* ♀ × *miliaris* ♂, is much more clearly shown in the cross *acutus* ♀ × *esculentus* ♂, species in which the characters of the plutei are so similar that the hybrid cannot be distinguished with certainty from the pure forms. We have therefore been

led chiefly to study the vesicle formation in this cross, on which a few words will be added below.

In 1912 we have obtained the remaining crosses with *miliaris*. Of these, we find that *esculentus* ♀ × *miliaris* ♂ behaves normally, with no elimination; *miliaris* ♀ × either *esculentus* or *acutus* ♂ shows only a small percentage of developing eggs, of which a considerable proportion at least show elimination of one, or at most a few chromosomes. We do not feel able to say with confidence whether these are paternal or maternal; in the few examples which are at the right stage of division, the length of the eliminated chromosomes suggests that they are paternal. We cannot, however, correlate this result with any confidence with the observation that the advanced plutei show maternal characters, for as Shearer, De Morgan and Fuchs have recorded, in 1912 the crosses with *miliaris* ♀ have shown a different behaviour from that of previous years, and have been as a rule of the paternal rather than of the maternal type. It is not certain, therefore, that our results obtained in 1912 are similar to what would have been obtained in 1911 or previous years. It is worthy of note, however, that in the cross *esculentus* ♀ × *miliaris* ♂, which in 1912, as in other years, has given purely maternal plutei, no chromosome elimination of any kind has been found.

With regard to vesicle-formation little need be said here, for the matter is thoroughly discussed in a paper by one of us published concurrently with this. We assumed at first that the chromosomes which develop vesicles are paternal, and that vesicle-formation is to some extent comparable with the elimination of paternal chromosomes described by Baltzer. Experiment with hypertonic solutions on eggs of the pure species, however, has made it very probable that the vesicles in the eggs of the cross *acutus* ♀ × *esculentus* ♂ are derived from the *acutus* (maternal) chromosomes, due probably to an alteration of the permeability or osmotic condition of the egg consequent upon the development within it of a foreign spermatozoon. Baltzer gives evidence on several

distinct lines that the chromosomes eliminated in his experiments were all paternal, so that if he is correct, it is probable that the vesicle-formation in our case is quite a different phenomenon. Tennent,¹ on the other hand, finds that both paternal and maternal chromosomes are eliminated in the cross *Arbacia* ♀ by *Toxopneustes* ♂, so that it is possible that elimination is not due simply to an incompatibility between the chromosomes of one species and the egg-cytoplasm of the other, but that, as suggested above, the physical condition of the cytoplasm is altered by the development within it of a foreign sperm-nucleus, causing the non-division or other abnormal behaviour of certain chromosomes, of either one or both species in different cases.

In any case a point of importance is that all these experiments give evidence of a physiological differentiation among the chromosomes. Some behave normally, others form vesicles, or fail to divide, and from the comparative constancy of the numbers it may be inferred that it is the same chromosomes in each case which are affected. We do not feel confident in identifying the chromosomes which form vesicles in the *acutus* ♀ × *esculentus* ♂ cross, but our general impression is that some at least of the affected chromosomes are the same in each case, suggesting that they differ in physiological characters as they do in shape and size. The physical cause of the vesicle-formation has been discussed by one of us elsewhere, but we put forward one additional suggestion which may possibly be worthy of consideration. Vesicles are produced in *acutus* chromosomes when the eggs have been treated with hypertonic solutions and have been returned to normal sea-water. It seems possible that the hypertonic solution withdraws water from the egg, causing it to have a higher concentration, both in the cytoplasm and nucleus, than the normal. At about the time the chromosomes are forming the egg is returned to water of lower concentration; the cytoplasm will then absorb water, and its concentration will thus be lower than that of the

¹ 'Journ. Exp. Zool.' xii, 1912, p. 397.

chromosomes lying in it. If, then, each chromosome can be regarded as a closed semipermeable membrane containing substances at a higher concentration than the surrounding cytoplasm now possesses, the chromosomes will absorb water, and in some cases swell so much as to produce vesicles.

In the cross *acutus* ♀ × *esculentus* ♂ the vesicle formation could be explained on the same hypothesis, if the *esculentus* spermatozoon takes up more fluid from the cytoplasm and especially from the egg-nucleus in forming the male nucleus than does the *acutus* sperm. It will act like the hypertonic solution in withdrawing water from the rest of the egg. The egg-cytoplasm will replace this water from the surrounding sea-water, but the chromosomes which have formed meanwhile in the egg-nucleus will have a higher concentration than the normal, and, as in the case of the eggs treated with hypertonic solutions, will show a tendency to swell and form vesicles. That the chromosomes may be regarded as being each enclosed in a semipermeable membrane is suggested by their normal behaviour in the late anaphase, when each chromosome swells up to form a vesicle in appearance like a small nucleus. Evidence in the same direction has been recently brought forward by A. A. Lawson¹, though we doubt whether his conclusions are entirely applicable to our cases. The suggestion that in the cross *acutus* ♀ × *esculentus* ♂, the *esculentus* sperm acts like a hypertonic solution in withdrawing water from the egg-nucleus is supported by the fact, referred to in our account of the conjugation of the nuclei, that in this cross the sperm-nucleus conjugates with the egg-nucleus while it is still quite small and contains a compact mass of chromatin, and while the chromosomes of the egg-nucleus are not yet definitely visible. In the converse cross, on the other hand, in which no vesicles are formed, the sperm-nucleus becomes nearly as large as the egg-nucleus before the two come into contact, so that conjugation does not

¹ Lawson, "Nuclear Omosis as a Factor in Mitosis," 'Trans. Roy. Soc. Edin.,' vol. xlvi, pt. i, 1912, p. 137.

take place until both nuclei are fully formed and contain visible chromosomes. It is doubtful, however, whether this can be the complete explanation of the vesicle-formation, for without further qualification it does not provide a reason for the production of vesicles in the second segmentation division. That the vesicle-formation is a phenomenon depending on the disturbance of the osmotic relations between the chromosomes and cytoplasm seems nevertheless a hypothesis which should be taken into account.

EXPLANATION OF PLATES 28 AND 29,

Illustrating Mr. L. Doncaster and Mr. J. Gray's "Cytological Observations on the Early Stages of Segmentation of *Echinus* hybrids."

[The figures were drawn with a Zeiss apochromat, 3 mm., n.a. 1.40 and with compens. o.c. 12. They are not all drawn to the same scale, figs. 5, 13, 14, 20, 22, 23, 29, 30 being on a smaller scale, figs. 8 and 17 on a larger scale than the rest. Since the chromosomes on the spindles are at different levels in the section, it was found that drawing by eye was preferable to using a camera. In some cases where the cell outlines are drawn, the nuclei or spindles are enlarged relatively to the cell.]

PLATE 28.

Fig. 1, *a*, *b*.—*E. acutus* ♀ × ♂. Two anaphase groups in face, from different eggs, 38 chromosomes in each. The two chromosomes outside the group to the left in 1 *b* belong to the other end of the spindle.

Fig. 2, *a*, *b*.—*E. esculentus* ♀ × ♂. Two anaphase groups in face, from the same spindle. Thirty-eight chromosomes in 1 *b*. 37 or 38 in 1 *a*.

Fig. 3, *a*, *b*.—*E. esculentus* ♀ × ♂. Anaphase (second division); spindle seen sideways. 3 *a* and 3 *b* are from successive sections; 38 at each end.

Fig. 4.—*E. esculentus* ♀ × *acutus* ♂. Anaphase, second division, in face; 38 chromosomes.

Fig. 5.—*E. esculentus* ♀ × *acutus* ♂. Anaphase, second division, side view.

Figs. 6-20.—*Acutus* ♀ × *esculentus* ♂.

Fig. 6, *a*, *b*.—*Acutus* ♀ × *esculentus* ♂. Two sections of prophase; 38 chromosomes, no vesicles at this stage.

Fig. 7.—Later prophase; formation of vesicles. The line round the spindle in figs. 6 and 7 represents the edge of the clear area in which the spindle lies.

Fig. 8, *a*-*z*.—Various shapes of chromosomes during vesicle formation. Early stages, first segmentation division.

Fig. 9, *a*, *b*.—Two sections of a late prophase, showing chromosomes beginning to divide and vesicles.

Fig. 10, *a*, *b*.—Anaphase, first division, showing vesicles left on equator of spindle, and divided poles. Some chromosomes added in both *a* and *b* from the next sections.

Fig. 11, *a*, *b*.—Later anaphase—only two vesicles. Five chromosomes added in 11 *a*, from the next section.

Fig. 12.—Telophase, showing normal chromosomes becoming vesicular at the poles; one large vesicle included among them; seven eliminated. Combined from two sections.

PLATE 29.

Fig. 13.—Beginning of cell-division. Several vesicles eliminated. Combined from two sections.

Fig. 14.—Completion of cell-division. Several vesicles eliminated.

Fig. 15, *a*, *b*.—Late prophase showing considerable division of poles.

Fig. 16, *a*, *b*.—Extreme case of division of poles giving quadripolar spindle. Not all the chromosomes are shown; they were not more than 38.

Fig. 17, *a*, *b*, *c*.—Vesicle-formation in second division.

Fig. 18 *a*, *b*.—Early anaphase, second division; two sections of one spindle. There were also one small chromosome and three small vesicles in the next section.

Fig. 19, *a*, *b*.—Anaphase, second division. Two sections of one spindle. The small vesicles on the lower edge of 19 *b* were added from the next section. In 19 *a* one long chromosome is displaced by the razor.

Fig. 20.—Telophase, second division. Three vesicles eliminated on each spindle.

Fig. 21, *a*, *b*.—*E. miliaris* ♀ × ♂. Two anaphase groups in face, from one spindle; 34 chromosomes at each end.

Figs. 22, 23.—*Acutus* ♀ × *miliaris* ♂. First division, showing narrow spindles, and in fig. 22, two vesicles. Not all the chromosomes shown.

Figs. 24, 25.—*Acutus* ♀ × *miliaris* ♂. Two spindles showing small vesicles. Fig. 25 combined from two sections.

Fig. 26, *a, b, c*.—*Acutus* ♀ × *miliaris* ♂. Chromosomes in three successive sections of a monaster; 36 chromosomes.

Fig. 27, *a, b*.—*Acutus* ♀ × *miliaris* ♂. Two anaphase groups in face, from one spindle; 37 chromosomes in 27 *a*, 31 in 27 *b*.

Fig. 28.—*Esculentus* ♀ × *miliaris* ♂. Anaphase group in face; 36 chromosomes.

Fig. 29.—*Miliaris* ♀ × *esculentus* ♂. Telophase, first division. Irregular groups at poles, three or four chromosomes eliminated. Combined from four sections.

Fig. 30.—*Miliaris* ♀ × *acutus* ♂. Two-cell stage. (Each cell was in metaphase of second division; section through the poles.) Three chromosomes eliminated (one drawn from the next section).